Listing of Claims:

- 1. (Currently amended) An in vitro method of activating protein kinase B comprising
- (a) obtaining from an insulin-responsive cell a membrane fraction, which comprises PDK2 ("phosphoinositide-dependent kinase 2") activity, and a cytoplasmic fraction, which comprises PDK1 ("phosphoinositide-dependent kinase 1") activity and which comprises a protein kinase B,
- (b) <u>preparing an assay mixture comprising combining</u> the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride,
 - (c) optionally adding a phosphatidylinositol phosphate compound to the assay mixture,

wherein (e) the protein kinase B is activated in the assay mixture by virtue of having a threonine residue phosphorylated and a serine residue phosphorylated, such that (d) the activated protein kinase B is capable of phosphorylating a GSK3 ("glucose synthase kinase-3").

- 2. (Original) The method of claim 1 wherein the insulin-responsive cell is treated with insulin.
- 3. (Original) The method of claim 2 wherein the membrane fraction is a plasma membrane fraction.
- 4. (Original) The method of claim 1 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.
- 5. (Currently amended) The method of claim 1 further comprising the step of combining PIP3 ("phosphatidylinositol 3,4,5-triphosphate") or PI(3,4)P2 ("phosphatidylinositol 3,4-biphosphate") with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.
- 6. (Original) The method of claim 5 further comprising the step of combining PIP3 with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.
- 7. (Original) The method of claim 1 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.

- 8. (Original) The method of claim 1 wherein the insulin-responsive cell is an adipocyte.
- 9. (Currently amended) An in vitro method of activating protein kinase B comprising
- (a) obtaining from an insulin-responsive cell a plasma membrane fraction and a cytoplasmic fraction, which comprises a protein kinase B,
- (b) treating said plasma membrane fraction with a solution comprising at least 145 mM chloride, thereby obtaining a salt-extracted plasma membrane fraction and an aqueous fraction,
- (c) desalting the aqueous fraction thereby producing a desalted aqueous fraction comprising less than 145 mM chloride,
- (d) <u>preparing an assay mixture comprising combining</u> the salt-extracted plasma membrane fraction, the cytoplasmic fraction, the desalted aqueous fraction, ATP, and a phosphatidylinositol phosphate molecule in a buffer comprising less than 145 mM chloride, wherein
- (e) the protein kinase B is activated <u>in the assay mixture</u> by virtue of having a threonine residue <u>phosphorylated</u> and a serine residue <u>phosphorylated</u> and a serine residue phosphorylated, such that
 - (d) the activated protein kinase B is capable of phosphorylating a GSK3.
- 10. (Original) The method of claim 9 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.
- 11. (Original) The method of claim 9 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.
- 12. (Original) The method of claim 9 wherein the insulin-responsive cell is an adipocyte.
- 13. (Original) The method of claim 9 wherein the insulin-responsive cell is treated with insulin.
- 14. (Original) The method of claim 9 wherein the phosphatidylinositol phosphate molecule is a PIP3 or PI(3,4)P2.
- 15. (Original) The method of claim 9 wherein the phosphatidylinositol phosphate molecule is a PIP3.

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16-21. (Canceled).

22. (Original) The method of claim 1 further comprising the step of combining PIP3 or PI(3,4)P2 with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.

23-29. (Canceled).